

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 69665/6857	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/US 00/ 40824	International filing date (day/month/year) 06/09/2000	(Earliest) Priority Date (day/month/year) 09/09/1999
Applicant ZYMETX, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,



the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,



the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.



as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/40824

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K31/70 A61P31/16		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) CHEM ABS Data, EPO-Internal, BIOSIS, WPI Data, EMBASE, PAJ, SCISEARCH, MEDLINE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 18324 A (LOEB LAWRENCE A ;MULLINS JAMES I (US); UNIV WASHINGTON (US)) 7 May 1998 (1998-05-07) page 4, line 5 - line 17 page 6, line 3 - line 7 page 7, line 31 - line 32 page 8, line 27 <div style="text-align: center; margin-top: 20px;"> --- -/-- </div>	1-19
<div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div>		
* Special categories of cited documents :		
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>*&* document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search	Date of mailing of the international search report	
19 March 2001	26/03/2001	
Name and mailing address of the ISA	Authorized officer	
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Brunnauer, H	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/40824

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KOJIC-PRODIC, BISERKA ET AL: "Conformation and structure of 2-amino-8-(.beta.-D- ribofuranosyl)imidazo '1,2-a!-s-triazin-4-one (5-aza-7- deazaguanosine), a potent antiviral nucleoside" BIOCHIM. BIOPHYS. ACTA (1982), 698(2), 105-10 , XP000984543 page 105; figure 1 page 106 page 109 ---	1-19
A	KOBE, B. ET AL: "Use of distance geometry approach for the in vitro antiviral activity evaluation of N-bridgehead C-nucleosides" EUR. J. MED. CHEM. (1992), 27(3), 259-66 , XP000984068 page 261; table 1 page 264 -page 265 ---	1-19
A	BLANCO, J. M. ET AL: "Synthesis and antiviral and cytostatic activities of carbocyclic nucleosides incorporating a modified cyclopentane ring. 1: Guanosine analogues." NUCLEOSIDES & NUCLEOTIDES, (1997) VOL. 16, NO. 1-2, PP. 159-171. , XP000984524 page 159 -page 160 page 163; table 2 -----	1-19

Information on patent family members

PCT/US 00/40824

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9818324 A	07-05-1998	AU 5095998 A	22-05-1998
		EP 0948256 A	13-10-1999
		US 6063628 A	16-05-2000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/17518 A2

(51) International Patent Classification⁷: **A61K 31/00**

(21) International Application Number: **PCT/US00/40824**

(22) International Filing Date:
6 September 2000 (06.09.2000)

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:
60/153,156 9 September 1999 (09.09.1999) **US**

(71) Applicant (for all designated States except US):
ZYMETX, INC. [US/US]; Suite 100, 800 Research
Parkway, Oklahoma City, OK 73104-3600 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **OJWANG, Joshua,**
Odoyo [KE/US]; 1313 N.W. 100 9th Street, Edmond, OK
73003 (US).

(74) Agents: **SAMPLES, Kenneth, H. et al.**; Fitch, Even,
Tabin & Flannery, Suite 1600, 120 South LaSalle Street,
Chicago, IL 60603 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— Without international search report and to be republished
upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **METHOD OF TREATMENT OF INFLUENZA**

(57) Abstract: The present invention discloses a method of treating a subject diagnosed or suspected of having an influenza virus infection. The method includes administering to the subject a pharmaceutically effective amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one or a pharmaceutically acceptable salt of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one. This method can also be used prophylactically for subjects who may be exposed to influenza virus.

WO 01/17518 A2

METHOD OF TREATMENT OF INFLUENZA

FIELD OF THE INVENTION

This invention relates to a method of treating influenza virus infections. The method includes administering an isomer of guanosine, or a
5 pharmaceutically acceptable salt thereof, to a subject infected with influenza virus.

BACKGROUND OF THE INVENTION

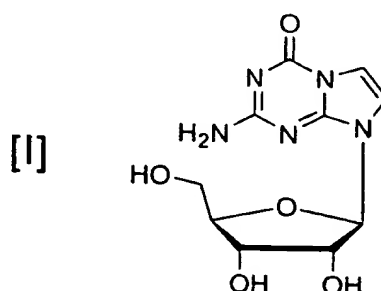
Viral infections are a principal cause of illness due to communicable diseases that affect the public at large. Of these, influenza viruses, including
10 types A and B, are a significant factor responsible for causing respiratory symptoms as well as systemic malaise; other respiratory viruses include parainfluenza 1, 2, 3, and 4, respiratory syncytial virus, and adenovirus. The influenza viruses undergo rapid mutation of strains, producing pathogens with varying degrees of virulence and severity of symptoms. Recently, influenza
15 infection has been as high as the fifth leading cause of death from acute respiratory disease in the United States (Morbidity and Mortality Weekly Report, 36 (1987) 2).

Influenza virus types A, B, and C belong to the family of Orthomyxoviridae. Influenza A and B are significant pathogens in children
20 and adults causing sever lower respiratory tract disease, whereas influenza C can cause sporadic upper respiratory tract illness. Influenza virus is highly contagious and can affect large proportions of the population each winter. Influenza A epidemics occur about every 2-3 years, whereas influenza B epidemics appear every 4-6 years. Symptoms include moderate to high fever
25 together with chills, headache, myalgia, rhinorrhea, among others. Importantly, virus progeny are detectable 24 hours prior to the appearance of symptoms, and virus titers peak 24-48 hours after symptoms arise.

For this reason it is important to have available ways of treating an influenza infection. Particularly among infants, the elderly, and those having

compromised or deficient immune responses, early treatment of influenza could minimize the risk of severe illness, and of morbidity.

U.S. Patent 4,246,408 discloses the use of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one [I]



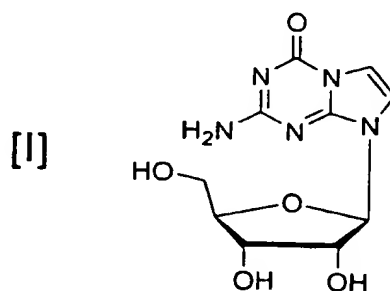
5 and related compounds as an antiviral agent for RNA type viruses. The imidazo[1,2-a]-s-triazine ring system may be regarded as a 5-aza-7-deazapurine. As such, compound [I] is an isomer of guanosine in which two of the atoms in the heterocyclic aromatic ring of the base have been
interchanged. An *in vitro* cell culture assay was adapted in which the
10 cytopathic effect (CPE) was used as an indicator of antiviral activity (Sidwell et al., Applied Microbiology 22:797-801 (1971)). Results are expressed as a virus rating having the broad ranges of (a) slight or no activity, (b) moderate activity, and (c) marked activity. It was found that compound [I] was markedly active against vesicular stomatitis virus, coxsackie B-1 virus and Echo-6 virus,
15 and moderately active against five rhino viruses. The skilled artisan recognizes that these varied levels of antiviral activity against the different viruses tested were not predicted or suggested before the experiments were done. We are not aware of any use or attempt to use compound [I] as an influenza treatment.

20 There remains a need for an effective method for the antiviral treatment of influenza viral infections. There additionally remains a need for a convenient method for treating influenza viral infections involving oral administration of a solid or liquid dosage form, or for the parenteral

administration of a liquid dosage form. The present invention recognizes these needs and addresses them.

SUMMARY OF THE INVENTION

The present invention discloses a method of treating a subject
5 diagnosed or suspected of having an influenza virus infection. The method includes administering to the subject a pharmaceutical dosage form that contains sufficient 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one (compound [I]), or pharmaceutical acceptable salts of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one, to be effective to treat an
10 influenza viral infection. Compound [I] has the following chemical formula:



In an important embodiment of the method, the pharmaceutical dosage form is a tablet, caplet, or capsule, and additionally includes one or more pharmaceutically acceptable stabilizers and excipients. In a further, significant embodiment of the method, the pharmaceutical dosage form is an
15 aqueous liquid, and the dosage form further contains one or more pharmaceutically acceptable stabilizers and excipients. In this embodiment, the liquid may be administered either orally or parenterally. In an additional important embodiment the dosage form is administered at least once daily over a time period of about three to about seven days. The amount of
20 compound [I] included in each dosage form should be sufficient to maintain pharmaceutical effectiveness in treating the influenza infection over the entire time period. Of course, the dosage amount can be adjusted or varied as desired to account for the body weight of the patient, severity of influenza, the

desired number and frequency of dosage, the desired total duration of treatment, and similar factors.

Thus, one object of the present invention is to provide a method of treating a subject diagnosed to be, or suspected of being, infected with influenza virus, said method comprising administering to the subject a dosage form containing a pharmaceutical effective amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one or a pharmaceutically acceptable salt of 2-amino-8-(β -D-ribofuranosyl)imidazo- [1,2-a]-s-triazin-4-one.

Another object is to provide a method of treating a human subject diagnosed to be, or suspected of being, infected with influenza virus, said method comprising administering to the subject a formulation containing a pharmaceutical effective amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one or a pharmaceutically acceptable salt of 2-amino-8-(β -D-ribofuranosyl)imidazo- [1,2-a]-s-triazin-4-one, wherein the formulation is administered to the subject one to four times per day and for a period of at least three days and wherein the amount administered is in the range of about 0.5 to about 60 mg/kg body weight per day.

Still another object of the present invention is to provide a method of reducing the risk of a human subject, who may be exposed to influenza virus, from becoming infected with influenza virus, said method comprising administering to the subject a pharmaceutical effective amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one or a pharmaceutically acceptable salt of 2-amino-8-(β -D-ribofuranosyl)imidazo- [1,2-a]-s-triazin-4-one, wherein the pharmaceutical effective amount is daily administered to the subject before the exposure or immediately after the exposure and then continued for at least five days after the exposure.

These and other objects and advantages of the present invention will be apparent from a consideration of the present application.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1. Dose-response curves describing the percent survival of MDCK cells infected with influenza A/Shangdong/09/93 (H3N2) when treated with compound [I] (solid curve, closed symbols) compared with cells treated with ribavirin (dot-dashed curve, open symbols).

Figure 2. Dose-response curves describing the percent survival of MDCK cells infected with influenza A/PR/8/34 (H1N1) when treated with compound [I] (solid curve, closed symbols) compared with cells treated with ribavirin (dot-dashed curve, open symbols).

Figure 3. Dose-response curves describing the percent survival of MDCK cells infected with influenza B/Hong Kong/5/72 when treated with compound [I] (solid curve, closed symbols) compared with cells treated with ribavirin (dot-dashed curve, open symbols).

Figure 4. Dose-response curves describing viral titer when MDCK cells infected with influenza A/Shangdong/09/93 (H3N2) were treated with compound [I] (solid curve, closed symbols) compared with cells treated with ribavirin (dot-dashed curve, open symbols).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of treatment of a subject having, or suspected of having, an influenza infection. The subject may be a human or other mammal; preferably, the subject is human. The method of treatment involves administering an effective amount of the compound 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one (compound [I]) or a pharmaceutically acceptable salt thereof to the subject. The objective of the treatment is to attenuate or ameliorate the severity of the influenza infection in the subject, and ultimately to eliminate the influenza infection completely. The efficacy of the treatment is reflected in its ability to accomplish such attenuation or amelioration. The amelioration or attenuation of the infection may include such beneficial effects as reduction of symptoms that may accompany the infection, including, but not limited to fever, myalgia, malaise,

and inflammation of the upper or lower respiratory tract. Without wishing to be limited by theory, it is believed that such attenuation or amelioration is accomplished because compound [I] interferes with, reduces, or inhibits the ability of the virus to replicate and/or produce progeny virus particles in the infected subject.

The synthesis of compound [I] is provided in U.S. Patent 4,246,408 and in references cited therein, all of which are incorporated herein by reference.

Compound [I] or pharmaceutically acceptable salts thereof are incorporated into any of a variety of pharmaceutical dosage forms known in the pharmaceutical sciences. Solid dosage forms include, by way of nonlimiting example, tablets, gell caps, caplets, and capsules. These solid dosage forms may further include any one or more pharmaceutically acceptable stabilizers and excipients. Stabilizers are included in order to impart chemical and physical stability to the active agent and to the dosage form itself, such that its shelf life is preserved to an extent that fulfills regulatory requirements and consumer needs. Excipients are included as required to extend the mass of the dosage form, to provide general chemical or physical properties required, and for other purposes leading to acceptability of the dosage form.

Liquid dosage forms include formulations intended for oral dosing and/or formulations intended for parenteral dosing. These liquid dosage forms are generally based on aqueous solutions including compound [I] or a pharmaceutically acceptable salt thereof, and may further include any one or more pharmaceutically acceptable stabilizers and excipients. The specific identity of such stabilizers and excipients may differ, depending upon whether the liquid is an oral or a parenteral dosage form. As with solid dosage forms, stabilizers are included in liquid dosage forms in order to impart chemical, physical, and microbiological stability to the active agent and to the entire liquid dosage form as a whole, such that its shelf life is preserved to an extent that fulfills regulatory requirements and consumer needs. Excipients are

included as required to provide general chemical or physical properties required, and for other purposes leading to acceptability of the dosage form. Preparation of pharmaceutical dosage forms is described in detail in Remington's Pharmaceutical Sciences, Martin, E.W., ed., latest ed., Mack Publishing Co., Easton, PA, which is incorporated herein by reference.

The solid dosage form is preferably administered by oral ingestion. Additionally, an advantageous liquid dosage form is likewise administered orally. Furthermore, an alternative formulation of a liquid dosage form may be administered parenterally, for example by hypodermic injection or by intravenous infusion. Either dosage form could, if desired, contain conventional additives or medicants to further reduce influenza symptoms. Additionally, the dosage form could contain colorants, flavorants, and the like.

For purposes of this invention, a "pharmaceutically acceptable salt" is intended to include those salts which are, within the scope of sound medical practice and judgement, suitable for use in humans and/or lower animals without undue toxicity, irritation, allergic response, and the like and provide a medically reasonable risk/benefit ratio. Of course, to be useful in the present invention, such pharmaceutically acceptable salts of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one should provide a similar benefit, but not necessarily equal to or better than, as provided by the free compound. Pharmaceutically acceptable salts are well known in the art.

A subject to be treated may be a human or other mammal suspected of having an influenza infection, or identified as having an influenza infection. Presentation with symptoms of influenza infection, including, but not limited to fever, myalgia, malaise, and inflammation of the upper and/or lower respiratory tract, may lead a knowledgeable specialist such as a physician or veterinarian to suspect that the subject has an influenza infection. Alternatively, analysis of a clinical sample provided by the subject may lead to a diagnostic result positively identifying the presence of influenza virus in a subject. Any such subjects may be treated by the methods of the present invention. Likewise, the methods of this invention may be used to reduce the

risk of a subject being infected with influenza. Thus, these methods might be used to treat, for example, family members or other such groups (e.g., nursing home residents) when one member is diagnosed with influenza. This method could also be used to treat subject who are at special risk to influenza and/or complications resulting from influenza; such treatments could begin before the typical flu season begins and continued through the flue season. Such prophylactic use could be used in combination with annual flu shots.

As used herein, administering an amount of compound [I] that is sufficient to be effective, or that maintains pharmaceutical effectiveness, in treating an influenza viral infection relates to providing amounts of the drug, over an appropriate dosing interval, that demonstrably attenuates the severity of the symptoms and/or shortens the duration of the disease. It is believed that 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one interferes with or inhibits the ability of influenza virus to replicate and/or to produce progeny virus particles. Pharmaceutical effectiveness relates to providing sufficient amounts of compound [I] to achieve attenuation and/or amelioration of the symptoms ascribed to influenza, such as fever, myalgia, malaise, and inflammation of the upper or lower respiratory tract. Attenuation relates to slowing or reducing the appearance of these symptoms. Amelioration relates to accelerating recovery from these symptoms once they have appeared. It is believed that these effects are due to the inhibitory effect of the drug on viral replication.

Any of the solid or liquid dosage forms defined above are to be used in the method of treating the influenza infection. It is shown in the Examples that compound [I] provides effective antiviral activity when tested in *in vitro* cell culture assays for interfering with or inhibiting influenza infection of the cells. The concentrations at which this effect is observed are generally at levels that may be conveniently incorporated into a dosage form of the invention and administered to a subject. Depending on the amount of compound [I] incorporated into a particular individual dosage form, the frequency of dosing is at least once a day, and may be twice, three, or more

times daily. Likewise, the duration of treatment may be one day, such as the day on which a diagnosis of influenza infection is made or the day on which a knowledgeable specialist may suspect the presence of an influenza infection (either in an individual or a group of closely associated individuals – especially when such persons are in a high risk group). Alternatively, the interval over which dosing is continued may extend for several days, such as an interval over which it is known that influenza infections generally endure. Additionally, the interval may be chosen to extend for a certain period beyond the length of time that an influenza infection generally endures, in order to ensure the complete effectiveness of the anti-influenza treatment method of the invention.

In general, the amount of compound [I] included in a particular dosage form, the number of times per day that the dosage form is administered, and the duration of this regime of dosing are all determinable by routine clinical and diagnostic methods such that the dosing is effective to attenuate, ameliorate, and ultimately to eliminate the influenza infection in the subject. In general, doses of compound [I], or its pharmaceutical acceptable salts, ranging from about 0.1 to about 750 mg/kg body weight per day may be used. Preferred doses are about 0.5 to about 60 mg/kg body weight per day; even more preferred are doses of about 1 to about 20 mg/kg body weight per day.

Treatment is preferably commenced before, at the time of, or as soon as possible after, infection and continued at least until the virus is no longer detectable in the respiratory tract. A suitable treatment regime, for example, would consist of one to four doses per day for at least three days, and preferably for about five to about seven days, post-infection. Of course, longer treatment durations can be used and, in some cases, will be preferred.

It is shown in the Examples that compound [I] effectively reduces the morbidity of cells in tissue culture infected with any of three strains of influenza A or influenza B tested. Compound [I] is also effective in reducing the titer of influenza virus present in cultures of infected cells. In contrast, it was found that no effect was observed when compound [I] was tested against

cells infected with a rhinovirus, a parainfluenza III virus, a respiratory syncytical virus, and an adenovirus. It is believed that the prior art offers no indication of the selectivity of the effectiveness of compound [I] with respect to influenza, and its lack of effectiveness against several other viruses. The effectiveness of compound [I] against influenza virus is therefore unexpected by a worker in the field of clinical virology.

The following examples are intended to illustrate the invention and not to limit it.

Example 1. Effect of compound [I] on cells infected with a strain of influenza A. A cytopathic effect (CPE) assay was undertaken using MDCK cells infected with influenza A/Shangdong/09/93 (H3N2). The MDCK cells were obtained from American Type Culture Collection (Manassas, Virginia). The cells were cultured in a minimum essential medium and incubated at 37°C in a 5% CO₂ atmosphere. Seven one-half log₁₀ dilutions of compound [I] were added to cell monolayers; the virus was added within 5 minutes and the plates were sealed for incubation. Positive control experiments were run in parallel using ribavirin (1-(β-D-ribofuranosyl)1,2,4-triazole-3-carboxamide) instead of compound [I]. About 72 to 168 hours after infection, neutral red dye was added to the medium. Cells not damaged by virus take up a greater amount of the dye. The amount of dye take up by the test cells or the positive control cells was read using a computerized microplate autoreader (EL308, Biotek-Instrument Inc., Winooski, Vermont). An effective concentration for inhibition of a virally-induced cytopathic effect is indicated as a concentration of the drug providing 50% diminution in cytopathicity (IC₅₀).

The results are shown in Figure 1. It is seen that compound [I] inhibited influenza A-induced cytopathicity in a dose dependent fashion, with an IC₅₀ of 6.8 µg/mL. The positive control, ribavirin had an IC₅₀ of 18 µg/mL.

Reference Example 1. Effect of compound [I] on cells infected with various viruses. The inhibition by compound [I] of a cytopathic effect induced by rhinovirus (strain Norman), parainfluenza III (strain 14702), respiratory syncytial virus (strain A2), and adenovirus (strain G5089) was separately

tested in analogous fashion as in Example 1. Compound [I] was inactive against these viruses.

Example 2. Effect of compound [I] on cells infected with a different strain of influenza A. Evaluation of the antiviral effect of compound [I] against another strain of influenza A (influenza A/PR/8/34 (H1N1)) was conducted using the same procedure as in Example 1. The results, shown in Figure 2, indicate that compound [I] inhibits influenza A/PR/8/34 in a dose dependent fashion. In this experiment compound [I] was slightly less active than ribavirin. The IC_{50} values were 13 and 5.6 $\mu\text{g/mL}$ for compound [I] and ribavirin, respectively.

Example 3. Effect of compound [I] on cells infected with influenza B/Hong Kong/5/72. To evaluate the effect of compound [I] on an influenza B virus, Hong Kong/5/72 strain was used. The experiment was again conducted using the CPE assay system in MDCK cells. The results are shown in Figure 3. It is seen that compound [I] inhibits influenza B/Hong Kong/5/72 in a dose dependent fashion. In this experiment compound [I] was slightly less active than ribavirin. The IC_{50} values were 16 and 5.2 $\mu\text{g/ml}$ for compound [I] and ribavirin, respectively.

Example 4. Anti-Influenza A Effect Using Viral Yield Assay System. Compound [I] was evaluated for antiviral activity in a viral yield assay. Influenza A/Shangdong/09/83 (H3N2) virus was used in the assay system. Briefly, the growth medium was decanted and the various dilutions of compound [I] were added to each well (6 wells/dilution, 0.1 mL/well). Four wells for each dilution of compound [I] and ribavirin were test wells to which virus was added, and 2 wells for each dilution of the compounds were toxicity control wells in which cells were present but to which no virus was added. Virus dilution medium was also added to cell control wells, to which no compound was added, at 0.1 mL/well. Virus was added approximately 5 min. after compound addition. The plates were sealed with plastic wrap (Saran™) and incubated at 37°C in a humidified incubator with 5% CO_2 , 95% air

atmosphere until virus control wells had adequate cytopathic effect (CPE) readings. This was achieved after 72 hours.

Cells were then examined microscopically for CPE, this being scored from 0 (normal cells) to 4 (maximal, 100% CPE). The cells in the toxicity control wells were observed microscopically for morphologic changes attributed to cytotoxicity. This observed cytotoxicity is graded at T (100% toxicity, complete cell sloughing from plate), P_{vh} (80% cytotoxicity), P_h (60% cytotoxicity), P (40% cytotoxicity), P_{si} (20% cytotoxicity), and 0 (normal cells). The medium and the cells from each test well were then harvested and all replicate samples were pooled. After one cycle of freeze/thaw to break up the cells, the medium and the cells from each test well were assayed for virus titer by inoculating varying 10-fold dilutions into microplate wells containing established monolayers of MDCK cells. Each dilution was assayed in triplicate. The plates were incubated at 37°C until read for viral CPE at three and six days after inoculation of samples. Virus titers at each level of compound treatment were determined and the log of selected titers bracketing the 90% reduction value (one log below the virus control titer) was plotted versus the respective compound concentrations. A curve was fitted to the graphed points and the formula of the fitted curve was used to calculate the concentration of compound providing 90% reduction of virus titers when compared with the titer of the virus controls. This concentration was designated the IC_{90} value (inhibitory concentration giving 90% reduction in virus titer). The IC_{50} and 50% cytotoxic concentration (CC_{50}) were calculated by regression analysis of the virus CPE data and the toxicity control data, respectively. The selectivity index (SI) for each compound was calculated by the formula $SI = CC_{50}/IC_{50}$. The known antiviral compound, ribavirin (1-B-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), was run in parallel with compound [I].

The results for the viral titer assay are given in Figure 4. The inhibition curve found for compound [I] is very unusual, because the reduction of the viral titer was not fully dose-dependent. The best effect was observed at the

middle of the concentration range tested (1-10 $\mu\text{g/mL}$). In the same experiment, ribavirin reduced the viral titer in a well-behaved dose dependent manner. The data show, nevertheless, that the IC_{90} values for compound [I] and ribavirin are comparable. The results for the pharmacological parameters defined above are tabulated in the following Table:

Table. Antiviral activity of compound [I]

	IC_{90} , $\mu\text{g/mL}$	CC_{50} , $\mu\text{g/mL}$	IC_{50} , $\mu\text{g/mL}$	SI
Compound [I]	7.5	>316	6.8	>4.6
Ribavirin	8.5	>100	18	>5.6

CLAIMS

We claim:

1. A method of treating a subject diagnosed to be, or suspected of being, infected with influenza virus, said method comprising administering to the subject a dosage form containing a pharmaceutical effective amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one or a pharmaceutically acceptable salt of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one.
2. The method described in claim 1, wherein the dosage form is a solid dosage form and wherein the dosage form further comprises one or more pharmaceutically acceptable stabilizers and excipients.
3. The method described in claim 2, wherein the solid dosage form is a tablet, caplet, or capsule.
4. The method described in claim 2, wherein the dosage form is administered at least once daily over a time period of at least about three days and wherein the amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one in each dosage form is sufficient to maintain pharmaceutical effectiveness over the time period.
5. The method described in claim 4, wherein the dosage form is administered one to four times daily and the time period is at least five days.
6. The method described in claim 5, wherein the amount administered is from about 0.1 to about 750 mg/kg body weight per day.

7. The method described in claim 6, wherein the amount administered is from about 0.5 to about 60 mg/kg body weight per day
8. The method described in claim 7, wherein the amount administered is from about 1 to about 20 mg/kg body weight per day.
9. The method described in claim 1, wherein the pharmaceutical dosage form is an aqueous liquid and wherein the dosage form further comprises one or more pharmaceutically acceptable stabilizers and excipients.
10. The method described in claim 9, wherein the liquid is administered orally or parenterally.
11. The method described in claim 10, wherein the dosage form is administered at least once daily over a time period of at least about three days and wherein the amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one in each dosage form is sufficient to maintain pharmaceutical effectiveness over the time period.
12. The method described in claim 11, wherein the dosage form is administered one to four times daily and the time period is at least five days.
13. The method described in claim 12, wherein the amount administered is from about 0.1 to about 750 mg/kg body weight per day.
14. The method described in claim 13, wherein the amount administered is from about 0.5 to about 60 mg/kg body weight per day
15. The method described in claim 14, wherein the amount administered is from about 1 to about 20 mg/kg body weight per day.

16. A method of treating a human subject diagnosed to be, or suspected of being, infected with influenza virus, said method comprising administering to the subject a formulation containing a pharmaceutical effective amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one or a pharmaceutically acceptable salt of 2-amino-8-(β -D-ribofuranosyl)imidazo- [1,2-a]-s-triazin-4-one, wherein the formulation is administered to the subject one to four times per day and for a period of at least three days and wherein the amount administered is in the range of about 0.5 to about 60 mg/kg body weight per day.

17. The method described in claim 16, wherein the formulation is administered to the subject for a period of at least five days and wherein the amount administered is in the range of from about 1 to about 20 mg/kg body weight per day.

18. A method of reducing the risk of a human subject, who may be exposed to influenza virus, from becoming infected with influenza virus, said method comprising administering to the subject a pharmaceutical effective amount of 2-amino-8-(β -D-ribofuranosyl)imidazo[1,2-a]-s-triazin-4-one or a pharmaceutically acceptable salt of 2-amino-8-(β -D-ribofuranosyl)imidazo-[1,2-a]-s-triazin-4-one, wherein the pharmaceutical effective amount is daily administered to the subject before the exposure or immediately after the exposure and then continued for at least five days after the exposure.

19. The method described in claim 18, wherein the amount administered is in the range of about 0.5 to about 60 mg/kg body weight per day.

1/2

Figure 1

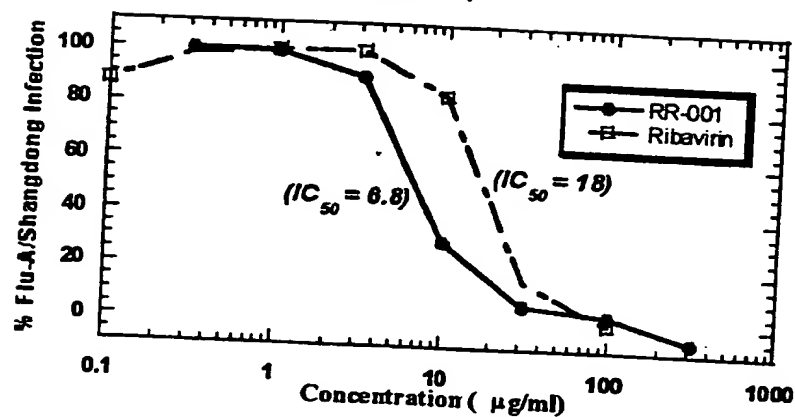
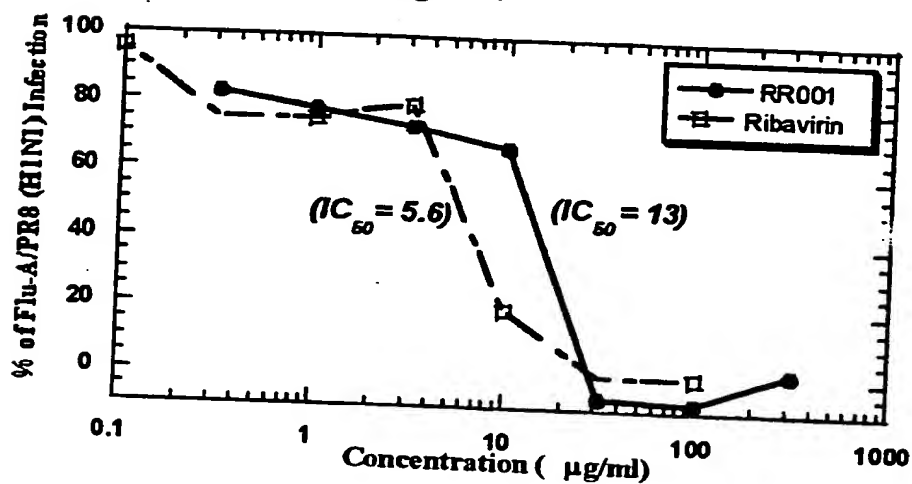


Figure 2



2/2

Figure 3

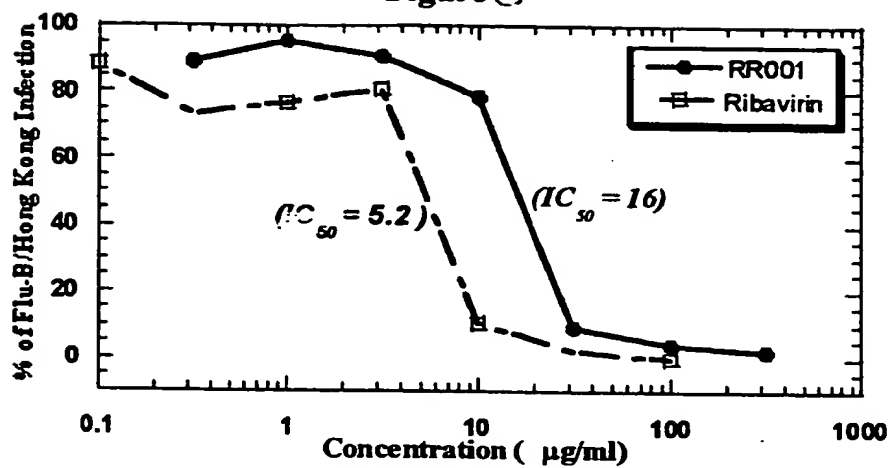
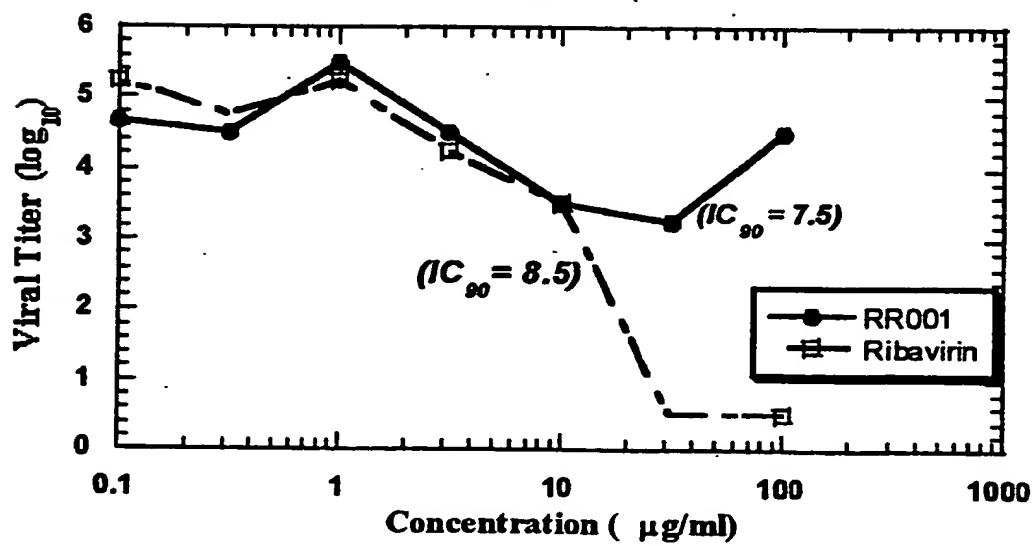


Figure 4



09/807770

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
15 March 2001 (15.03.2001)

PCT

(10) International Publication Number
WO 01/17518 A3

(51) International Patent Classification⁷: **A61K 31/70.**
A61P 31/16

(21) International Application Number: PCT/US00/40824

(22) International Filing Date:
6 September 2000 (06.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/153.156 9 September 1999 (09.09.1999) US

(71) Applicant (for all designated States except US):
ZYMETX, INC. [US/US]; Suite 100, 800 Research
Parkway, Oklahoma City, OK 73104-3600 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **OJWANG, Joshua,**
Odoyo [KE/US]; 1313 N.W. 100 9th Street, Edmond, OK
73003 (US).

(74) Agents: **SAMPLES, Kenneth, H.** et al.; Fitch, Even,
Tabin & Flannery, Suite 1600, 120 South LaSalle Street,
Chicago, IL 60603 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW). Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM). European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE). OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

(88) Date of publication of the international search report:
11 October 2001

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: METHOD OF TREATMENT OF INFLUENZA

(57) Abstract: The present invention discloses a method of treating a subject diagnosed or suspected of having an influenza virus in-
fection. The method includes administering to the subject a pharmaceutically effective amount of 2-amino-8-(β-D-ribofuranosyl)im-
idazo[1,2-a]-s-triazin-4-one or a pharmaceutically acceptable salt of 2-amino-8-(β-D-ribofuranosyl)imidazo-[1,2-a]-s-triazin-4-one.
This method can also be used prophylactically for subjects who may be exposed to influenza virus.

RECEIVED

NOV 06 2001

TECH CENTER 100/2908



WO 01/17518 A3

INTERNATIONAL SEARCH REPORT

h. national Application No

PCT/US 00/40824

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/70 A61P31/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, BIOSIS, WPI Data, EMBASE, PAJ, SCISEARCH, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 98 18324 A (LOEB LAWRENCE A ;MULLINS JAMES I (US); UNIV WASHINGTON (US)) 7 May 1998 (1998-05-07) page 4, line 5 - line 17 page 6, line 3 - line 7 page 7, line 31 - line 32 page 8, line 27</p> <p style="text-align: center;">--- -/--</p>	1-19

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

19 March 2001

Date of mailing of the international search report

26/03/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040. Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Brunnauer, H

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 00/40824

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>KOJIC-PRODIC, BISERKA ET AL: "Conformation and structure of 2-amino-8-(.beta.-D- ribofuranosyl)imidazo '1,2-a!-s-triazin-4-one (5-aza-7- deazaguanosine), a potent antiviral nucleoside" BIOCHIM. BIOPHYS. ACTA (1982), 698(2), 105-10 , XP000984543 page 105; figure 1 page 106 page 109</p>	1-19
A	<p style="text-align: center;">---</p> <p>KOBE, B. ET AL: "Use of distance geometry approach for the in vitro antiviral activity evaluation of N-bridgehead C-nucleosides" EUR. J. MED. CHEM. (1992), 27(3), 259-66 , XP000984068 page 261; table 1 page 264 -page 265</p>	1-19
A	<p style="text-align: center;">---</p> <p>BLANCO, J. M. ET AL: "Synthesis and antiviral and cytostatic activities of carbocyclic nucleosides incorporating a modified cyclopentane ring. 1: Guanosine analogues." NUCLEOSIDES & NUCLEOTIDES, (1997) VOL. 16, NO. 1-2, PP. 159-171. , XP000984524 page 159 -page 160 page 163; table 2</p> <p style="text-align: center;">-----</p>	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/40824

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9818324 A	07-05-1998	AU 5095998 A	22-05-1998
		EP 0948256 A	13-10-1999
		US 6063628 A	16-05-2000
<hr/>			